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Table of Contents

		Page
I.	**Introduction**	
	Purpose	2
II.	**Body**	
	Experimental methods	2
Study I -	Evaluation of total energy expenditure (doubly labeled water) across different measurement periods during arduous work	3
Study II -	Energy balance and water turnover during arduous wildland fire suppression	21
Study III -	Seasonal variations in total body water from multifrequency bioelectrical impedance and deuterium dilution in the wildland firefighter	38
III.	**Conclusions**	54
IV.	**References**	56

I. Introduction

Purpose

The purpose of this extension project was to complete three studies with male and female subjects during short term and seasonal exposure to an arduous work environment and to determine the effects of an uncontrolled situation (active wildland firefighters engaged in wildfire suppression) and gender on measures of total energy expenditure (TEE), energy balance, total body water and body composition. Although prior research has been performed using simulated field operations (military field training) or with specific sport competitions, it was the purpose of our studies to apply a variety of measurement techniques to an unpredictable field environment where the subjects were exposed to all feasible physical and psychological stress associated with the job. The three investigations that have been completed as part of this grant are discussed in the order of completion below (I, II and III).

Study I - **Evaluation of total energy expenditure (doubly labeled water) across different measurement periods during arduous work**

Study II - **Energy balance and water turnover during arduous wildland fire suppression**

Study III - **Seasonal variations in total body water from multifrequency bioelectrical impedance and deuterium dilution in the wildland firefighter**

II. Body

Experimental Methods

Study I - **Evaluation of total energy expenditure (doubly labeled water) across different measurement periods during arduous work**

Abstract

The purpose of this investigation was to determine the total energy expenditure (TEE) using the doubly labeled water (DLW) methodology during two measurement periods (1-3 and 4-5 days). We have previously reported the TEE in a small sample of wildland firefighters during a five-day experimental period of arduous wildfire suppression (MSSE 31(5): s366, 1999). We hypothesized that day to day variations in work assignments may influence the range of EE during a five-day work assignment. Seven additional wildland firefighters were included with the 5 original subjects (n=6 M, 6 F). Prior to wildland fire suppression, each subject was given an oral dose of ^2H_2O and $H_2{}^{18}O$ (approximately .23g ^2H_2O/kg TBW, .39g $H_2{}^{18}O$/kg TBW). Urine samples were collected between 0400-0600 daily. Samples were analyzed using isotope ratio mass spectrometry for ^{18}O and ^2H enrichment and fitted using a linear elimination curve for each measurement period. Isotope elimination, subsequent CO_2 production and TEE were calculated using days 1-3 and 1-5. Using a weighted average for the short and long measurement periods, average TEE for days 4-5 was extrapolated. Data were analyzed using a dependent t-test. TEE was 4150±888 and 4176±1636 kcal/24 hours during days 1-3 and4-5, respectively. The energy expenditure associated with physical activity (EEA) was calculated assuming dietary thermogenesis at 10% of TEE (TEEx.9-BMR). Calculated EEA was 2105±709 and 2128±1445 kcals/24 hours for days1-3 and 4-5, respectively. Considering the original values calculated from the five day elimination (TEE=4160±1012 kcals/24 hours, EEA=2115±846 kcals), demonstrates consistent daily energy expenditure in the wildland firefighter.

Introduction

Previous research has indicated that the doubly labeled water (DLW) methodology serves as the gold standard for the measurement of total energy expenditure (TEE) of free living individuals (Schoeller, 1999; Westerterp, 1999). However, the methodology requires rigorous quality control in regards to isotope preparation, application and timing of the dose and the collection of samples (Westerterp, 1999). These issues make the use of the doubly labled water methodology more difficult during field operations. However, several studies have used this technique during field operations including military operations (Jones, 1993; Hoyt, 1991, 94; Burstein, 1996; Mudambo, 1997; Delany, 1989; Forbes-Ewan, 1989), mountainous/arctic expedition (Westerterp, 2000; Pulfrey, 1996; Stroud, 1993), jungle multiple stage bicycle racing (Westerterp, 1986), extended training (Sjodin, 1994; Trappe, 1997), and space travel (Stein, 1999; Lane, 1997). In the last decade, the use of doubly labeled water for measurement of TEE has received notable acceptance as the gold standard. However, the majority of prior "field" use of DLW has been conducted on a previously determined schedule. Previous protocols have been somewhat predictable in terms of the start and finish times of the experiment.

Past research that has been done in a variety of field settings has used DLW for measurement periods of 5-8, (Hoyt, 1994; Westerterp 86; Forbes-Ewan, 89; Trappe, 97), 10-12 (Jones, 93; Burstein, 96, Mudambo, 97, Hoyt, 91). Because the accuracy of the calculated TEE is dependent on the elimination rates (kH and kO) of both isotopes, it is necessary for the measurement period to be long enough to accurately calculate the difference in $^{2}H_2O$ and $H_2{}^{18}O$. In the sedentary or recreationally active individual, this may require 10-14 days given the typical

isotopic dose (approximately 0.12 $g \cdot kg^{-1}$ total body water for ^2H_2O and approximately 0.3 $g \cdot kg^{-1}$ total body water for $H_2{}^{18}O$). However, when the work environment is likely to result in high rates of water turnover, the measurement period can be shortened significantly so long as adjustments are made to the initial isotopic dose to accommodate the more rapid rates of elimination.

Interestingly, to our knowledge, the inclusion of females in previous field operations research has been minimal. Trappe (1997) measured the TEE of elite female swimmers while training at the U.S. Olympic Training Center. Westerterp et. al included two (1992) and four females (1994) during high altitude mountaineering expeditions on Mt. Sajama, Bolivia and on Mt. Everest. Trappe (1997) noted TEE values of 5593±495 $kcals \cdot day^{-1}$ during five days of 2 swim $sessions \cdot day^{-1}$, lasting a total of 5-6 $hours \cdot day^{-1}$.

During the summer months in the western part of the United States, land management agencies (United States Forest Service, Bureau of Land Management, State Forestry) are involved in wildfire suppression activities. Wildland fire suppression is a seasonal occupation requiring long hours of heavy work under adverse conditions (extended work shifts up to 24 hours, high ambient heat, and compromised dietary intake, smoke inhalation, altitude exposure). The common wildland firefighting tasks often include hiking, fireline construction, chain saw work and brush removal and typically average approximately 7.5 $kcals \cdot min^{-1}$. If a 3:1 work to rest ratio (45:15 minutes each hour) is assumed, for a 12-14 hour work day, work shift energy expenditure may exceed 4050 to 4725 kcals·12 and 14 $hours^{-1}$, respectively. However, this data has been collected during "simulated" fireline activity using douglas bags and indirect calorimetry.

Although extremely accurate, the use of indirect calorimetry for extended measurement periods on the fireline is not practical. In addition, the use of DLW allows for an extended measure of TEE during an "un-simulated" wildfire suppression assignment provided issues related to dose delivery, initial isotope equilibration, and sample collection strategies can be accommodated.

The wildland firefighter is often subjected to high levels of physiological stress which is often coupled with psychological stress, compromised nutritional intake and extreme environmental conditions (altitude and high ambient heat). Consequently, the purpose of this study was to use the DLW methodology to determine the TEE in male and female wildland firefighters during short term (3-7 days) arduous wildfire suppression.

Methods

Subjects

Subjects included male (n=7) and female (n=10) wildland firefighters recruited from three Interagency Hot Shot Crews (Lolo, Bitterroot, St. Joe, Sierra crews) from Western Montana and Idaho. Subjects were recruited through an informative meeting arranged between the Principal Investigator and all Interagency Hot Shot Crew Supervisors prior to the 1997 and 1998 fire seasons. An informational meeting was then arranged between the Principal Investigator and the entire crew. At this time, the objectives of the study and the outline of data collection was discussed. Potential subjects were selected and were tested upon deployment during five different fire assignments in Montana, California, Florida, Washington and Idaho.

Preliminary Screening

Prior to data collection, all subjects read and signed an Internal Review Board (IRB) approved human subject's consent. Subjects completed a detailed health history to determine prior exercise and training habits and menstrual regularity.

Isotope administration

Upon arrival to the incident, subjects were provided with an oral dose of doubly labeled water (0.39g/kg BW $H_2{}^{18}O$ (10% APE), 0.23g/kg BW ^2H_2O (99.9% APE), Cambridge Isotope Laboratories, Andover, MA; Isotec, Inc., Miamisburg, OH). Subjects were given the initial dose of doubly labeled water upon arrival to the incident in the evening hours prior to initiating fire suppression activity (approximately 2200 hours). The protocol for the dosing and the daily collection schedule is detailed below in Figure I-1.

Although the initial dose is somewhat higher (for $H_2{}^{18}O$) compared to estimates suggested by Wolfe, 1992 (0.25g/kg BW $H_2{}^{18}O$, 0.3g/kg BW ^2H_2O), a larger dose is warranted to compensate for the anticipated higher rates of water turnover. All overnight voids were collected to correct the measure of total body water (TBW).

Day	*-1*	*0*	*1*	*2*	*3*	*4*	*5*	*5*	*6*
Collection time	2200	0630	0430	0430	0430	0430	0430	2200	0630
Dose Information									
0.39g $H_2{}^{18}O$, 0.23g ^2H_2O/kg BW	*								
2.0 g ^2H_2O								*	
Urine Sampling information									
background	*							*	
urine – first void			*	*	*	*	*		
urine – second void		*							*
Calculations									
Total body water (TBW)		TBW							TBW
Total energy expenditure (TEE) - long		<			TEE – long period			>	
Total energy expenditure (TEE) - short		<	TEE - short period	>					

Figure I-1. Dose and daily collection schedule for measures of TEE using the doubly labeled water technique.

Samples were collected and stored in 5 ml cryogenic vials on ice for the duration of the experimental period. TBW was calculated from the change in isotopic enrichment (background vs. the second void urine) using equation 1. In addition to the initial dose of doubly labeled water, a second dose of ^2H_2O (approximately 2.0g) was administered on the eve of day 5 after the collection of a second background sample. The following morning, a first void and second urine samples were collected. All overnight voids were collected to correct the measure of TBW. TBW was calculated as previously mentioned. First void samples were collected on mornings 1, 2, 3, and 5 for isotopic analyses and calculation of TEE during approximately 72 and 120 hours of wildfire suppression activity.

Body composition was calculated pre and post fire suppression from TBW (calculated from ^2H_2O dilution) in each subject during the 1997 and 1998 seasons. During the 1998 season, skinfold measures were also measured (n=12). Skinfolds were collected in rotational order according to the gender specific formulas of Jackson and Pollock (1978, 1981). No less than three independent site measurements were obtained until repeat measurements were within ±1 mm. Because the original prediction equations of Jackson and Pollack (1978, 1981) were developed with the Lange skinfold calipers, an adjustment of +2mm for each site was included to compensate for differences noted for the Harpenden calipers (Golding et. al, 1989). Body density was converted to percent body fat using an appropriate age and gender equation of Lohman (1992). Body composition estimated from the TBW values (calculated from ^2H_2O dilution, corrected for overnight void collections). FFM was calculated as TBW/.73. FBM was calculated from the difference in the nude body weight and the calculated FFM.

Experimental Fire Suppression Period

During deployment to five different wildfire incidents (MT, CA, FL, WA, ID), subjects were studied during five consecutive days of wildfire suppression. Additional subjects that did not receive the isotopic dose were also studied during the same period for adjustments in background abundances due to a change in the geographic location and water source. Wildfire suppression assignments commenced shortly after the collection of the second void sample on day 1 (see Figure 1). Wildfire suppression included extensive hiking with a load, fire line construction with a pulaski (modified axe for ground scraping), chain saw work and brush removal. Work shifts ranged from 12-18 hours during the entire experimental period on all wildfire assignments. The experimental period was separated into short (approximately 72 hours) and long (approximately 120 hours) segments. The rationale for this was to determine if there was variation in the calculated average TEE during the initial attack portion of wildfire suppression as compared to the five day segment which was noted as having less active work shifts on days four and five. Typically, the Interagency Hot Shot crews will work extended shifts during the initial stages of an assignment to gain control of the wildfire. However, after the fire is controlled (a fire line has been constructed around the entire perimeter), work shift energy expenditure declines as firefighters "hold" the line (monitoring the interior of the fire with some hiking but minimal had tool or saw work).

Isotopic analyses and TEE calculations

The Nutritional Sciences Laboratory at the University of Wisconsin, Madison, conducted isotopic analyses of all urine samples. Each urine sample was mixed with ca. 200 mg of dry carbon black and filtered through a 0.22 micron filter to remove particulate materials and much

of the organic material. Two 1mL aliquots of each specimen were placed in 2 mL septum sealed, glass vials. Deuterium analysis was performed by reducing 0.8uL of cleaned fluid over chromium at 850°C (Gehre et. al, 1997), which produces pure H_2 gas that is introduced to a Finnigan MAT Delta Plus isotope ratio mass spectrometer. Deuterium abundance was measured against a working standard using a standard dual inlet, Faraday Cup, differential gas isotope ratio procedure. Enriched and depleted controls were analyzed at the start and end of each batch and these secondary standards used to calculate the "per mille" abundance versus Standard Mean Ocean water for each urine sample. All analyses were performed in duplicate and all specimens from the same participant analyzed during the same batch. Results were corrected for any memory from the previous chromium reduction process. If duplicates differed by more than 5 per mil, duplicate analyses were repeated. The second aliquot was equilibrated with 1 mL (STP) of carbon dioxide at constant temperature (Schoeller, 1997). The CO_2 was removed by syringe and roughly 200 uL injected onto a 10 cm x 1/8" Chromasorb Q column. The CO_2 peak was introduced into the ion source of a Finnigan MAT Delta S isotope ratio mass spectrometer and the $^{18}O/^{16}O$ ratio measured under dynamic flow conditions. A secondary standard was injected at the start and end of each batch. The secondary standard was used to calculate the "per mille" abundance versus Standard Mean Ocean Water (SMOW) for each specimen. Analyses were performed in duplicate and all specimens from the same participant analyzed during the same batch. Results were corrected for any memory from the previous chromium reduction. If duplicates differed by more than 0.5 per mil, analyses were repeated in duplicate. Isotope dilution space was calculated as described by Coward and Cole (1992). Total body water was calculated by averaging the deuterium dilution space/1.041.

Total body water and water turnover were calculated using equations 1-5. CO_2 production and average daily TEE (kcals·24 hours^{-1}) was calculated using equations 6-8. The isotopic elimination rate for ^2H and ^{18}O were calculated using the two-point method for the short and long experimental periods corrected for changes in the baseline isotopic abundance from the control subjects on each incident. The food quotient (FQ) was calculated from the dietary intake records of each subject during the short and long experimental periods using equation 9. Generally, the FQ were lower than the estimated mixed diet value of .85 due to the higher fat intake of the current subjects.

Equation 1. Calculation of total body water from the change in isotopic enrichment .

$$\left[\text{TBW (kg)} = \frac{d}{MW} \bullet \frac{APE}{100} \bullet \frac{18.01}{R_{std} \bullet \Delta\delta^2} \right] / \quad 1.041$$

d =	isotopic dose in grams
MW =	the molecular weight of ^2H_2O (20.00)
APE =	atom percent excess of ^2H_2O stock solution (99.99)
18.01 =	molecular weight of unlabelled water
R_{std} =	isotopic difference noted in the standard (0.00015576)
$\Delta\delta^2$ =	change in enrichment from background (relative to SMOW) to second void
1.041 =	assumed isotope dilution space for ^2H_2O.

Equation 2. Elimination rate of ^2H_2O (k2)

$$k2 = \text{nlog}\ (\Delta\delta^2 / \Delta\delta^2 b) / \text{ days}$$

$\Delta\delta^2$ =	change in enrichment from background (relative to SMOW) to second void
$\Delta\delta^2 b$ =	change in enrichment from background to second void
days =	experimental period in days

Equation 3. Rate of gaseous water loss (rGH_2O)

$$\mathbf{rGH_2O = 1.45 \cdot Pre\ TBW \cdot (1.041 \cdot k2)}$$

1.45 = laboratory constant
Pre TBW = Pre experimental period total body water (moles)

Equation 4. Elimination rate of water (rH_2O) in moles$\cdot$day^{-1}

$$\mathbf{rH_2O\ (mol \cdot day^{-1}) = (Pre\ TBW \cdot k2)\ /\ f}$$

f = estimated fractionation factor
$[(.99 \cdot rGH_2O)/\text{Pre TBW} \cdot k2 \cdot 0.059)]$

Equation 5. Elimination rate of water (rH2O) in l$\cdot$day^{-1}

$$\mathbf{rH_2O\ (l \cdot day^{-1}) = (rH2O\ (mol \cdot day^{-1}) \cdot 18.015)\ /\ 1000}$$

18.015 = g$\cdot$mol^{-1} of H_2O

Equation 6. Difference in the elimination of the ^2H and ^{18}O

$$\mathbf{\Delta\ k = (1.007 \cdot k^{18}O) - (1.041 \cdot kD_2O)}$$

$$= (1.007 \cdot -.2179) - (1.041 \cdot -.1717)$$

$$\mathbf{= 0.0407}$$

Equation 7. **CO_2 production rate calculated from the average of the pre-experimental TBW from ^2H and ^{18}O dilution (extrapolated to AM void).**

$$\mathbf{rCO_2 = .455 \cdot \Delta\ k \cdot average\ TBW\ (moles)}$$

$$= .455 \cdot 0.0407 \cdot 2552.8$$

$$\mathbf{= 47.25\ moles/day}$$

Equation 8. **Calculated TEE in $kcals \cdot 24\ hours^{-1}$.**

$$\mathbf{TEE\ (kcal/day) = (VCO_2\ (1.1+3.9/FQ) \cdot 22.4)}$$

$$= 47.25 \cdot (1.1 + 3.9/.81) \cdot 22.4$$

$$\mathbf{= 6261\ kcals/day}$$

Equation 9. **Calculated food quotient (FQ) from the dietary intake records of each subject during the long and short experimental periods.**

$$\mathbf{FQ = (0.81 \cdot \%protein) + (0.71 \cdot \%fat) + (1 \cdot \%carbohydrate)}$$

$$= (0.81 \cdot 18.7\%\ prot) + (0.71 \cdot 35.2\%\ fat) + (1 \cdot 40.4\%\ carb)$$

The components of TEE were estimated from the summary equation TEE = DIT + BMR + EEA, where DIT is the thermogenic effect of the dietary intake during the measurement period (TEE x .1), BMR is basal metabolic rate and, EEA is the energy expenditure of physical activity. Basal metabolic rate (BMR) was calculated based on total body water ([21.6 • (TBW/.73)] + 370). Physical activity (EEA) was calculated from the known value of TEE and the calculated estimates of DIT and BMR. Values of TEE (kcal/24 hours and multiples of BMR) and EEA were calculated for the different measurement periods. Isotope elimination, subsequent CO_2 production and TEE were calculated using days 1-3 and 1-5. Using a weighted average for the short and long measurement periods, average TEE for days 4-5 was extrapolated.

Hydration Markers

In addition to TBW, select measures to identify acute changes in hydration status were measured including water turnover (rH_2O, as indicated above) and urine specific gravity and osmolality.

Urine specific gravity was measured using a hand held refractometer (Atago Uricon – NE, Farmingdale, NY). Prior to sample series analyses, a drop of distilled water was placed on the face of the prism. Using distilled water as the standard, the instrument was adjusted to 1.000. Each mixed second void sample was analyzed in duplicate. Urine osmolality was determined using freezing point depression (Precision Systems mOsmette Model 5004). Samples were analyzed in duplicate at all collection time points. Before any samples were analyzed, the osmometer was calibrated against standards of known osmolality (100, 500 mOsmol - CONTROL, Natick, MA).

Statistical Methods

All descriptive data (height and age) are expressed as mean±sd. A one way repeated measures ANOVA across time (pre vs. post) was used to determine differences in body weight and body composition (FFM and FBM) using both the ^2H_2O dilution (n=17) and skinfold methods) and for the hydration measures of urine osmolality and specific gravity. A 2x2 repeated measures ANOVA (measurement period (short vs. long) and intake vs. expenditure) was used to determine differences between the reported intake and expenditure and differences between TEE across the two measurement periods (kcals·24 hours^{-1}). A one way repeated measures ANOVA (short vs. long measurement periods) was used to determine variations in other measures of TEE (kcal·kg^{-1}·24 hours^{-1}, kcal·kg FFM^{-1}·24 hours^{-1}, TEE expressed as a function of BMR (x BMR). TEE of physical activity (EEA), EEA·kg^{-1}·24 hours^{-1}, EEA·kg FFM^{-1}·24 hours^{-1} and water turnover (rH_2O)).

Results and Discussion

Subject characteristics

Descriptive data for all subjects are presented in Table II-1. Although data was collected on 8 males and 9 females, one male from the 1997 season is not included in the D2O dilution results for body composition due to an error during the post experimental dosing period. Although there were no statistically significant decreases in body weight or changes in FFM or FBM, the females had a tendency to increase FFM and decrease FBM.

Table I-1. Descriptive data for the sample of wildland firefighters studied during the 1997-98 fire season experimental seasons.

Variable	MALES		FEMALES	
	Pre-fire	Post-fire	Pre-fire	Post-fire
Body Weight (kg)	74.6±6.4	74.1±7.2	65.2±8.0	65.3±7.6
ΔFFM (D2O dilution – kg)	65.4±7.0	64.8±8.4	49.2±4.9	50.2±5.7*
ΔFBM (D2O dilution – kg)	9.9±3.6	9.8±3.7	16.0±4.4	14.8±4.7†

*p=0.0529 vs. pre fire, †p=0.0616 vs. pre fire, Δ indicates (n=7 males, n=9 females)

As previously mentioned, TEE was determined using the doubly labeled water technique. Data for the measure of TEE was expressed using several units of measure. These results are shown in Table I-2. Although the males had a significantly higher rate of daily energy expenditure, there were no differences between the genders relative to total body weight or estimated basal metabolic rates. However, when TEE expenditure was expressed as a function of the energy expenditure associated with physical activity (EEA=TEE-BMR-DIT, where DIT is assumed at 10% of TEE and BMR is assumed at [21.6 • (TBW/.73)] + 370), the males had a significantly

higher rate of energy expenditure. Again, however, when this was expressed relative to total body weight, there were no differences between the males and females.

Table I-2. Total energy expenditure in the wildland firefighters studied during the 1997-98 fire season experimental seasons.

Variable	MALES	FEMALES	pvalue
TEE (kcal/day)	4878±716	3541±718*	0.0016
TEE (kcal/kg/day)	66.3±14.2	54.8±11.2	0.0815
TEE (xBMR)	2.8±.5	2.5±.5	0.2134
TEE (EEA)	2628±714	1754±625*	0.0167
TEE (EEA/kg)	36.0±12.3	27.2±9.7	0.1185

* $p<0.05$ vs. males

In the present investigation, our main objective was to determine the usefulness of the DLW method for varying measurement intervals by including linear fit analyses of days 1-3 and 4-5. The rationale for this comparison was that the wildland firefighters work assignments are not often consistent. It was our original hypothesis that low intensity work shifts/days may dilute the daily average for TEE when considering the original data based on a five-day average. Table I-3 shows the comparison for TEE across the two measurement periods. Although there were some differences across sex (likely attributed to variations in FFM and total body size), TEE was similar for each measurement period.

Table I-3. **Comparison of the total energy expenditure across the two measurement periods (days 1-3 and days 4-5). The data from the original analyses (days 1-5 have been included for comparion.**

Variable	MALES	FEMALES
TEE (kcal/day)		
Days 1-3	4616±639	3684±898*
Days 4-5	5032±1853	3320±836*
Days 1-5	4878±716	3541±718*
TEE (xBMR)		
Days 1-3	2.6±.3	2.5±.6
Days 4-5	2.7±.6	2.4±.5
Days 1-5	2.8±.5	2.5±.5
TEE (EEA)		
Days 1-3	2374±536	1836±804
Days 4-5	2748±1769	1509±738
Days 1-5	2628±714	1754±625*

* p<0.05 vs. males

In addition to the total energy expenditure data, total energy intake was estimated from dieary recall as described above. Total intake and the relative contribution of each macronutrient are reported in Table I-4 and I-5. Although the total intake was not statistically significant between the sexes, the males tended to eat more. Expressed as a percent of the total intake, the males consumed less carbohydrate, and more fat and protein in comparison to the females. When the dietary intake data was further analyzed to determine if adequate carbohydrate and protein had

been consumed so as to better maintain FFM, it was noted that although there were no differences between sexes, the total amount from carbohydrate sources was slightly lower that recommendations associated with this type of exercise/work (approximately 10 g/kg BW/day). Similarly, the protein intake was in excess of recommended amounts for this type of work/exercise (approximately 1.8 g/kg BW/day) for the males.

Table I-4. Total energy intake and the percent contribution of each macronutrient during the experimental periods.

Variable	MALES	FEMALES	pvalue
Total intake (kcal/day)	4068±939	3222±713	0.0523
Percent Carbohydrate	46.8±5.6	58.8±8.2*	0.0034
Percent Fats	35.8±4.2	27.9±7.9*	0.0223
Percent Protein	15.7±2.9	13.2±2.0*	0.0489

* p<0.05 vs. males

Table I-5 Total energy intake (g/kg BW/day) for carbohydrate and protein intake during the experimental periods.

Variable	MALES	FEMALES	pvalue
Carbohydrate	6.5±1.7	7.3±2.0	0.3930
Protein	2.2±.6	1.7±.5	0.0795

In addition to the total intake patterns during the experimental period, the intake patterns during the "post shift" hours were evaluated to determine if subjects were consuming adequate carbohydrate to ensure glycogen resynthesis. Over the post shift time period (average estimate = 5 hours), the carbohydrate intake averaged .62±.2 and .49±.2 g/kg/hour for the females and

males, respectively. Although the differences between sexes were not statistically significant ($p=0.2285$), these values are lower than what might be recommended for adequate glycogen re-synthesis post exercise (approximately 1 g/kg BW/hour).

Using the D2O elimination rates, the total water turnover was calculated to determine the hydration demands associated with the work stress. These data are included in Table I-6. Although there were no significant differences between the sexes, these values are extremely high and further emphasize the environmental stress associated with the occupation.

Table I-6. Total water turnover (L) during the wildfire suppression period.

Variable	MALES	FEMALES	pvalue
rH_2O (L/day)	7.3±1.2	6.7±2.0	0.4349
rH_2O (ml/kg BW/day)	98.8±17.1	101.8±22.6	0.7631

Although previous research has used the doubly labeled water methodology in the field (cycling (Westerterp et al., 1986), swimming (Trappe et al., 1997) military operations (Hoyt et al., 1991, 1994), mountaineering (Pulfrey et al., 1996; Westerterp et al., 1992, 1994)), previous protocols have been somewhat predictable in terms of start and finish times. However, the present study represents an attempt to study the application of the DLW technique in an unpredictable occupational setting (wildland fire suppression).

Previous studies have documented rates of energy expenditure at levels similar to those noted in the wildland firefighter. Hoyt has described the energy expenditure of Marines during simulated

combat/training scenarios approaching 5000 kcal/day. Similarly Trappe et al. (1997) documented rates of TEE as high as 5500 kcal/day in elite level female swimmers. Westerterp et al. (1986) has described the "energetic ceiling" for humans to be near 4-5 times basal metabolic rate (7000-9000 kcal/day). However, Westerterp et al. (1986) has also recognized the shortcomings of the DLW methodology during measurement periods with extreme body water turnover (isotopic fractionation issues) and the need to control for these.

The current data suggests that subjects were able to maintain energy balance with the self-selected energy intake. However, it was apparent that the energy intake for the males was significantly lower than the TEE. The usefulness of the DLW methodology is limited because of obvious expense, methodological issues surrounding sample analyses and calculation issues (isotopic fractionation). Regardless, the methodology is robust enough for use during unpredictable environmental conditions where water turnover (rH_2O) exceeds 8.5 liters/day so long as attempts are made to adjust for isotopic fractionation due to evaporative water loss. The major finding from this re-evaluation of the TEE data relates to the consistency of the values regardless of the measurement period (1-3 vs. 4-5 days). This represents a consistent day to day pattern of energy expenditure in the WLFF and or/the robust qualities of the DLW technique. Our original hypothesis was that by calculating TEE over the original measurement period (5 days), we were diluting the effects of the original wildfire suppression efforts. We anticipated that the values for TEE for days 1-3 (the initial 72 hours of the work assignment) would be somewhat higher in comparison to the values for days 4-5 (the later 48 hours of the work assignment). However, this was not what the data re-analyses demonstrated. In contrast to our original hypothesis, the male have a tendency towards higher rates of EE during the later 48

hours of suppression. Most importantly, these data demonstrate an arduous and consistent work environment for the WLFF. These data further suggest that this may serve as an ideal model to determine the physiological effects associated with arduous field conditions in males and females.

Study II - **Energy balance and water turnover during arduous wildland fire suppression**

Abstract

The purpose of this investigation was to determine the effects of wildfire suppression activity on the maintenance of energy balance and body composition in male and female wildland firefighters (WLFF). WLFF (n=14) were measured prior to and following a 5-day experimental period and compared to a control group (n=13) of recreationally active college students. Changes in total body weight, total body water and body composition were evaluated prior to an following the experimental period using ^2H_2O dilution and skinfold measures. Water turnover from the calculated rate of ^2H elimination (rH_2O) and urine measures of osmolality and specific gravity were also collected to determine the hydration demands of the job. Compared to controls, WLFF demonstrated a significant ($p<0.05$) decrease in total body weight (pre 71.9±10.4, post 70.9±10.2) and total body water (pre 42.9±7.2, post 42.0±6.7). Both the skinfold and the ^2H_2O dilution techniques demonstrated that WLFF lost a significant ($p<0.05$) amount of fat free mass (skinfold pre=60.4±7.8, skinfold post=59.0±10.0; ^2H_2O pre=59.8±7.2, ^2H_2O post=57.5±9.2 kg). However, there were no detectable decreases in the fat body mass from either measurement method. Control subjects maintained body weight and body composition. These results demonstrate an arduous work environment that often compromises energy balance. Collectively,

the decrease in body weight, total body weight and fat free mass may be due to compromised hydration, a change in glycogen status or a decrease in the protein component of the fat free mass.

Key Words: Total body water, body composition, occupational physiology, firefighting

Introduction

Previous research has indicated that the energy demands during field operations can routinely exceed in the upwards of 5000-6000 $kcals \cdot 24^{-1}$ based on the use of doubly labeled water (Mudambo 97, Hoyt 91, Stroud 93). Much of this research is conducted during military operations or during expedition (mountain or arctic). During the summer months in the western part of the United States, a variety of agencies (United States Forest Service, Bureau of Land Management, State Forestry) are involved in controlled burn operations and wildfire suppression.

Wildland fire suppression is a unique seasonal occupation that requires long hours of heavy work under adverse conditions (extended work shifts up to 24 hours, high ambient heat, compromised dietary intake, smoke inhalation, and altitude exposure). Based on a conservative estimate of energy expenditure for typical wildland firefighting tasks (7.5 $kcals \cdot min^{-1}$, 12-14 hour work shift), work shift energy expenditure may exceed 4050 to 4725 kcals·12 and 14 $hours^{-1}$, respectively (assuming 45 minutes of work each hour). Therefore, a simple job task analysis reveals that the energy demands of the job are extreme and represent a challenge to the maintenance of energy balance.

Our laboratory has recently determined the total energy expenditure during wildland fire suppression activities using the doubly labeled water and heart rate methodologies (Ruby 1999, Burks, 1998). Although there is variation in the calculated rates of TEE (dependent on fire location, work detail, amount of hiking and fire line construction), values range from approximately 3000 – 6500 kcals·24 hours^{-1} (Ruby, 1999). These previous data demonstrate a unique work environment that results in an abrupt increase in the required dietary intake patterns. Wildland firefighters are required to "self-adjust" to the increase in TEE within the restrictions of what is provided for them in the fire camp.

The adequacy of common food rations on the maintenance of energy balance was investigated during 12 days of military operations in the heat (African bush) (Mudambo et. al, 1997). Using the doubly labeled water and energy balance methods, TEE was calculated (5489±358 and 6205±167 kcals·24 hours^{-1} for the DLW and EB methods, respectively). During the 12-day period of combined heat stress and work, subjects lost 3.0±0.1 kg (from energy deficit and a decrease in total body water) indicating a deficiency in the standard food rations provided. Similar studies have demonstrated significant changes in energy balance in response to adverse field conditions in the cold (Delany, 1989), during progressive hypoxia during mountain expedition (Pulfrey, 1996; Westerterp, 2000) during extended training (Sjodin, 1994), and during space flight (Lane, 1997; Stein, 1999).

There is an inconsistent pattern within the previous research regarding an individuals ability to maintain energy balance during extreme field operations that result in TEE greater than 4,000

$kcal \cdot 24\ hours^{-1}$. However, the maintenance of energy balance is dependent on TEE and on the availability of foodstuffs in the field and consistent adequate intake behaviors. Regardless of availability, sustained or suppressed appetite will enhance and/or impede the maintenance of energy balance when matched with arduous field conditions.

Because the wildland firefighter is often subjected to unpredictable field stress during wildfire suppression, this research model represents an "un-simulated" work environment involving arduous muscular work coupled with physiological and psychological stress under extreme environmental conditions (altitude and ambient heat). Consequently, the purpose of this study was to determine the maintenance of energy balance and body composition in male and female wildland firefighters during a period of five days of arduous wildfire suppression.

Methods

Subjects

Subjects included wildland firefighters (N=14) recruited from four Interagency Hot Shot Crews (Lolo, Bitterroot, St. Joe, Sierra crews) from Western Montana, Idaho, and Northern California and recreationally active (N=13) University students. Subjects were recruited through an informative meeting arranged between the Principal Investigator and all Interagency Hot Shot Crew Supervisors prior to the 1997 and 1998 fire seasons. An informational meeting was then arranged between the Principal Investigator and the entire crew. At this time, the objectives of the study and the outline of data collection were discussed. Potential subjects were selected and were tested upon deployment to various fire assignments. Control subjects were recruited

through the undergraduate and graduate courses within the Department of Health and Human Performance.

Preliminary Screening

Prior to data collection, all subjects read and signed an Internal Review Board (IRB) approved human subject's consent. Subjects completed a detailed health history to determine prior exercise and training habits and menstrual regularity.

Total Body Water and Skinfold Measurements

Upon arrival to the incident, subjects were provided with an oral dose of ^2H_2O (approximately 2 grams- Cambridge Isotope Laboratories, Andover, MA) after the collection of a background urine sample (at approximately 2200). The ^2H_2O was mixed in 35 ml of tap water and was rinsed three times to ensure complete isotopic delivery. Subjects refrained from the consumption of food or additional water until first void urine samples were collected the following morning (approximately 0430). A second void urine sample was also collected at approximately 0600. Following the first void, a nude body weight was obtained (accuracy ±100 grams). All overnight voids were collected to correct the measure of total body water (TBW). Samples were collected and stored in 5 ml cryogenic vials on ice for the duration of the experimental period. TBW was calculated from the change in isotopic enrichment (background vs. the second void urine) using equation 1. Control subjects were studied on the University campus and reported to the laboratory at similar time points for the background and dosing protocol (approximately 2200) and the collection of first and second void samples (approximately 0600).

In addition to the measure of TBW and body composition from ^2H_2O dilution, skinfold measures were completed on each subject. Skinfolds were collected in rotational order according to the gender specific formulas of Jackson and Pollock (1978, 1981). No less than three independent site measurements were obtained until repeat measurements were within ±.1 mm. Because the original prediction equations of Jackson and Pollack (1978, 1981) were developed with the Lange skinfold calipers, an adjustment of +2mm for each site was included to compensate for differences noted for the Harpenden calipers (Golding et. al, 1989). Body density was converted to percent body fat using an appropriate age and gender equation of Lohman (1992). Body composition was also estimated from the TBW values (calculated from ^2H_2O dilution, corrected for overnight void collections). Fat free mass (FFM) was calculated as TBW/.73. Fat body mass (FBM) was calculated from the difference in the nude body weight and the calculated FFM.

A second dose of ^2H_2O was provided on the evening of day five following the collection of an additional background urine sample. The same procedures for isotopic dosing, urine collection, nude weight and skinfold measures were completed at this time to evaluate post experimental changes in body composition, TBW and body composition from both methods. Figure II-1 illustrates the protocol for data collection during wildfire deployment. Control subjects underwent the identical procedures with the exception of wildfire suppression. Control subjects were studied during the early part of the fall semester.

Day	-1	0		1	2	3	4	5	6	
Collection time		2200		0430	0430	0430	0430	0430	2200	0430
Dose Information										
2.0 g ^2H_2O		*							*	
Sampling information										
urine		†		(¥) 2nd void					†	(¥) 2nd void
Nude BW (kg)				x						x
Calculations										
Total body water (TBW)			TBW							TBW
				<	wildfire suppression work period				>	

Figure II-1. Isotopic administration protocol and wildfire suppression work period. * 2.0 gram ^2H_2O dose in 35 ml of tap water, † urine collection for background ^2H enrichment, ¥ second void urine collection for change in ^2H enrichment and for the calculation of TBW, x nude body weight measure ±100 grams after first void.

Isotopic analyses

The Nutritional Sciences Laboratory at the University of Wisconsin, Madison, conducted isotopic analyses of all urine samples. Breifly, each urine sample was mixed with ca. 200 mg of dry carbon black and filtered through a 0.22 micron filter to remove particulate materials and much of the organic material. Two 1mL aliquots of each specimen were placed in 2 mL septum sealed, glass vials. Deuterium analysis was performed by reducing 0.8uL of cleaned fluid over chromium at 850°C (Gehre et. al, 1997), which produces pure H_2 gas that is introduced to a Finnigan MAT Delta Plus isotope ratio mass spectrometer. Deuterium abundance was measured against a working standard using a standard dual inlet, Faraday Cup, differential gas isotope ratio procedure. Enriched and depleted controls were analyzed at the start and end of each batch and these secondary standards used to calculate the "per mille" abundance versus Standard Mean Ocean water for each urine sample. All analyses were performed in duplicate and all specimens from the same participant analyzed during the same batch. Results were corrected for any memory from the previous chromium reduction process. If duplicates differed by more than 5

per mil, duplicate analyses was repeated. The second aliquot was equilibrated with 1 mL (STP) of carbon dioxide at constant temperature (Schoeller, 1997). The CO_2 was removed by syringe and roughly 200 uL injected onto a 10 cm x 1/8" Chromasorb Q column. The CO_2 peak was introduced into the ion source of a Finnigan MAT Delta S isotope ratio mass spectrometer and the $^{18}O/^{16}O$ ratio measured under dynamic flow conditions. A secondary standard was injected at the start and end of each batch. The secondary standard was used to calculate the "per mille" abundance versus Standard Mean Ocean Water (SMOW) for each specimen. Analyses were performed in duplicate and all specimens from the same participant analyzed during the same batch. Results were corrected for any memory from the previous chromium reduction. If duplicates differed by more than 0.5 per mil, analyses were repeated in duplicate.

Isotope dilution space was calculated as described by Coward and Cole (1992). Total body water was calculated by averaging the deuterium dilution space/1.041 (see Equation 1 below).

Hydration Markers

In addition to TBW, select measures to identify acute changes in hydration status were measured including water turnover (rH_2O from ^2H_2O elimination following the initial dose throughout the experimental period), urine specific gravity and osmolality. Urine specific gravity and osmolality were measured using the second void samples collected on day zero and day six (the same samples used for the determination of ^2H_2O dilution).

Water Turnover ($rH^2{}_2O$)

Water turnover was calculated from the initial dose of ^2H_2O and the change in enrichment from the initial sample (2nd void on day 0) and the background sample collected on day 5 in

conjunction with an estimated factor to adjust for assumed fractionation. The elimination rate of ^2H_2O and rH_2O was calculated using equations 2-5 below.

Equation 1. Calculation of total body water from the change in isotopic enrichment .

$$\left[\textbf{TBW (kg)} = \frac{\textbf{d}}{\textbf{MW}} \bullet \frac{\textbf{APE}}{\textbf{100}} \bullet \frac{\textbf{18.01}}{\textbf{R}_{\textbf{std}} \bullet \Delta\delta^2} \right] \Big/ \quad \textbf{1.041}$$

d =	isotopic dose in grams
MW =	the molecular weight of ^2H_2O (20.00)
APE =	atom percent excess of ^2H_2O stock solution (99.99)
18.01 =	molecular weight of unlabelled water
R_{std} =	isotopic difference noted in the standard (0.00015576)
$\Delta\delta^2$ =	change in enrichment from background (relative to SMOW) to second void
1.041 =	assumed isotope dilution space for ^2H_2O.

Equation 2. Elimination rate of ^2H_2O (k2)

$$\mathbf{k2 = nlog\ (\Delta\delta^2 / \Delta\delta^2 b)\ /\ \ days}$$

$\Delta\delta^2$ =	change in enrichment from background (relative to SMOW) to second void
$\Delta\delta^2 b$ =	change in enrichment from background to second void
days =	experimental period in days

Equation 3. Rate of gaseous water loss (rGH_2O)

$$\mathbf{rGH_2O = 1.45 \bullet Pre\ TBW \bullet (1.041 \bullet k2)}$$

1.45 = laboratory constant
Pre TBW = Pre experimental period total body water (moles)

Equation 4. Elimination rate of water (rH_2O) in $moles \cdot day^{-1}$

$$\mathbf{rH_2O\ (mol \cdot day^{-1}) = (Pre\ TBW \bullet k2) / f}$$

f = estimated fractionation factor
$[(.99 \bullet rGH_2O)/Pre\ TBW \bullet k2 \bullet 0.059)]$

Equation 5. Elimination rate of water (rH2O) in $l \cdot day^{-1}$

$$\mathbf{rH_2O\ (l \cdot day^{-1}) = (rH2O\ (mol \cdot day^{-1}) \bullet 18.015) /\ 1000}$$

$18.015 = g \cdot mol^{-1}$ of H_2O

Urine Specific Gravity

Urine specific gravity was measured using a hand held refractometer (Atago Uricon – NE, Farmingdale, NY). Prior to sample series analyses, a drop of distilled water was placed on the face of the prism. Using distilled water as the standard, the instrument was adjusted to 1.000. Each mixed second void sample was analyzed in duplicate.

Urine Osmolality

Urine osmolality was determined using freezing point depression (Precision Systems mOsmette Model 5004). Samples were analyzed in duplicate at all collection time points. Before any samples were analyzed, the osmometer was calibrated against standards of known osmolality (100, 500 mOsmol - CON-TROL, Natick, MA).

Statistical Methods

Water turnover (rH_2O) was analyzed using a one way between subjects ANOVA. A two way (2 x 2) mixed design ANOVA (1 between - group, 1 within – time) was used to analyze the

variables of body weight, TBW, urine specific gravity, and osmolality. An additional 3 way (2 x 2 x 2) mixed design ANOVA (1 between – group, 2 within – methodology (^2H_2O and skinfold) and time (pre and post)) was used to evaluate changes in FFM and FBM from ^2H_2O dilution and the skinfold techniques. Results were considered statistically significant at the $p<0.05$ level. When statistically significant, interactions were analyzed with a series of multiple contrasts using the SuperAnova software package for the Macintosh.

Results

There was no significant difference in the average age of the subjects (29.3±4.7 and 25.5±6.0 for the WLFF and controls, respectively). Changes in total body weight, and total body water are shown in Table II-1. The WLFF showed a significant ($F=40.487$, $p=0.0001$) decrease in total body weight during the period of wildfire suppression. The WLFF also showed a significant ($F=9.706$, $p=0.0046$) decrease in total body water after wildfire suppression. However, there were no changes in total body weight and total body water in the control subjects. There were also observed differences between the controls and the WLFF subjects at each time point (see Table II-1).

Table II-1. Changes in body weight (kg) and total body water (kg) for the WLFF and control subjects. Data are expressed as mean±SD.

	Pre	*Post*
WLFF (n=14)		
Nude body weight (kg)	71.9±10.4†	70.9±10.2*†
Total body water (kg)	42.9±7.2†	42.0±6.7*†
Controls (n=13)		
Nude body weight (kg)	68.3±10.6	68.0±10.6
Total body water (kg)	37.8±6.2	37.9±6.3

*p<0.05 vs. Pre, † p<0.05 vs. Controls

Calculated rates of water turnover from the initial ^2H_2O dilution were significantly higher for the WLFF compared to the control subjects (F=40.391, p=0.0001). Rates of water turnover were 6.7±1.4 and 3.8±1.0 $l \cdot 24hrs^{-1}$ for the WLFF and controls, respectively.

Measures relating to hydration status of the subjects are presented in Table II-2. There was a significant main effect of group for the variable of urine specific gravity (F=7.019, p=0.0138). Values for specific gravity were significantly higher in the control subjects compared to the WLFF (1.017±0.006 and 1.023±0.007 for the WLFF and controls, respectively). There were no significant differences over time for either group. Similarly, there was a significant main effect for the variable of urine osmolality (F=4.295, p=0.0487). Values for urine osmolality were also significantly higher in the control subjects compared to the WLFF (596±196 and 751±244 for the WLFF and controls, respectively).

Table II-2. Changes in urine osmolality and specific gravity for the WLFF and control subjects. Data are expressed as mean±SD.

	Pre		*Post*
WLFF (n=14)			
Urine olsmolality (mOsmol)	1.016±0.006		1.018±0.0006
Urine specific gravity	562±175		629±216
rH_2O		6.7±1.4†	
Controls (n=13)			
Urine olsmolality (mOsmol)	1.024±0.007		1.022±0.007
Urine specific gravity	791±264		710±226
rH_2O		3.8±1.0	

† $p<0.05$ vs. controls

Changes in body composition calculated from the ^2H_2O dilution and skinfold methodologies are shown in Table II-3. For the measures of FFM, the group x time x method interaction was significant ($F=5.337$, $p=0.0294$). Multiple comparisons revealed that the WLFF showed a significant decrease in FFM from pre to post suppression activity for the skinfold ($F=24.492$, $p=0.0001$) and ^2H_2O ($F=58.292$, $p=0.0001$) methods. In contrast, the controls did not show a significant change in FFM from either methodology. There were also significant differences in the calculated FFM across the testing methods. The skinfold results estimated FFM significantly higher than ^2H_2O for the WLFF (post time point) and controls (pre and post time points).

For the measures of FBM, the group x time x method interaction was significant ($F=5.317$, $p=0.0297$). Multiple comparisons revealed that neither the WLFF nor the control subjects showed a significant change in FFM across time. However, at each time point, the skinfold calculation resulted in significantly lower estimates of FBM compared to the ^2H_2O dilution method.

Table II-3. Changes in calculated fat body mass (FBM) and fat free mass (FFM) from ^2H_2O dilution and skinfold. Data are expressed as mean±SD.

	Pre	*Post*
WLFF (n=14)		
FFM ^2H_2O dilution	59.8±7.2	57.5±9.2*¥
FFM skinfold	60.4±7.8	59.0±10.0*
FBM ^2H_2O dilution	13.1±5.0¥	13.4±5.2¥
FBM skinfold	11.5±7.7	11.0±7.5
Controls (n=13)		
FFM ^2H_2O dilution	51.8±8.5¥	51.9±8.7¥
FFM skinfold	54.0±8.2	53.6±8.2
FBM ^2H_2O dilution	16.5±6.0¥	16.0±6.3¥
FBM skinfold	14.3±5.7	14.4±5.9

* $p<0.05$ vs. Pre, † $p<0.05$ vs. Controls, ¥ $p<0.05$ vs. skinfold

Discussion

The purpose of this investigation was to determine the effects of unpredictable wildfire suppression work on the maintenance of energy balance in male and female WLFF subjected to

arduous work related stress. In addition, measures of hydration status and rates of water turnover from ^2H_2O dilution were calculated. The work environment for the wildland firefighter often requires an abrupt adjustment to changes in ambient temperature, altitude, exercise load and the necessary intake of food and water to maintain the energy demand of the job over many days. Using the doubly labeled water methodology, we have previously determined that the energy demands of the occupation vary by location and fire but may typically exceed between 3000 and 6000 kcals$\cdot$24 hrs^{-1} (Ruby et. al, 1999). In the present investigation a different group of male and female WLFF were studied in response to a similar acute period of wildfire suppression (5-7 days).

The results of this study indicate that the wildland firefighter has a tendency to lose total body weight and total body water during short-term wildfire suppression activity. The calculated rates of water turnover from ^2H_2O elimination indicate that the hydration demand of the job is extreme and amounts to a minimal ingestion of approximately 6-8 liters of water$\cdot$day^{-1}from beverages and other food sources. The range of rH_2O was 4.5-9.6 and 2.9-6.0 l$\cdot$min^{-1} for the WLFF and controls, respectively. However, this may represent a minimal level of intake as urinary measures of hydration status demonstrated subtle dehydration throughout the experimental period. Using two independent measures of body composition, it was noted that while the WLFF tended to maintain FBM, they lost FFM. Moreover, the two measures of body composition were in agreement indicating a consistent trend in FFM loss. In contrast, there were no differences noted in the control subjects for total body weight, FFM or FBM regardless of method.

Previous research has documented that the TEE associated with military related field operations is high and can compromise energy balance. Hoyt et. al (1994) studied six male soldiers during 5 days of strenuous winter exercise at moderate altitudes (2,500-3,100 m). Total energy expenditure from DLW was 4,558±566 kcals·day^{-1} with an average decrease in body weight of -1.8±0.5 kg. However, the slight decrease in total body water (-0.1±0.6kg) and FFM (-0.4±0.5 kg) was not significant. The loss in total body weight is not surprising given that the average caloric intake was approximately 2355 kcal·day^{-1} (representing an average daily deficit of 2200 kcal·day^{-1}). Other investigators (Burstein et. al 1996, Mudambo et. al, 1997) have also demonstrated inadequate intake patterns during field operations.

In contrast, Jones et. al (1993) demonstrated an overall maintenance of energy balance despite the apparent disparity between recorded intake (2633±499 kcal·day^{-1}) and TEE (4,317±927 kcal·day^{-1}). Although total body weight showed a significant decrease during the experimental period (-0.63±0.83 kg), there were no significant changes in FBM or FFM. Jones et. al (1993) suggested that the field rations provided did provide the adequate energy needs to maintain energy balance during a 10-day experimental period. These results are similar to Trappe et. al (1997) who demonstrated that female swimmers were able to maintain energy balance (based on minimal changes in total body weight; 65.4±1.6 and 65.2±1.5 kg for day 1 and day 5, respectively) despite an average TEE of 5593±495 kcal·day^{-1}. However, the changes in total body water during the experimental period were not reported.

In the current investigation, the WLFF subjects exhibited a negative energy balance based on changes in total body weight and FFM (considering both measurement techniques of ^2H_2O

dilution and skinfold). Interestingly, the mean decrease in nude body weight (1kg) was very similar to the decrease in total body water (0.9kg). This may simply indicate a change in total body hydration status as a result of the experimental period. Given the constraints for measuring body composition in a fire camp, measurement errors should also be considered. However, measurement error was minimized by having the same investigator conduct all skinfold measures, by the use of Harpenden calipers, and strictly adhering to the ±.1 mm repeatability for each site measure. Regardless, there was consistent agreement between both methodologies.

It is possible that the loss in body weight and total body water reflect a change in the muscle glycogen status. When the dietary intake patterns from our DLW studies (Ruby, 1999) are considered, diets were somewhat low in CHO (53±9%, range = 40 to 71%) and high in fat (32±7, range = 17-42%). The average daily CHO intake was 6.9 $g \cdot kg^{-1} \cdot day^{-1}$ and may under represent the typical daily CHO demand to maintain adequate liver and muscle glycogen stores for 12-16 hours of work. Previous research by Blomstrand and Saltin (1999) indicates that when initial glycogen levels are suppressed, the net rate of protein degradation is increased. Therefore, if muscle glycogen levels are not maintained, the increase in protein degradation may eventually compromise the protein component of the FFM.

The activity associated with wildland fire suppression may involve arduous hiking with a load (including a fire line pack (typically 11-16 kg), fire shelter (approximately 2 kg), and a Pulaski fire line tool (approximately 2 kg) or chainsaw (approximately 7-9 kg) and fire line construction (heavy digging or chainsaw work). Therefore, the active muscle mass is diverse and involves a

significant upper and lower body component. Therefore, whole body glycogen depletion is possible when the typical work shift often exceeds 12-16 hours·day^{-1}.

These results indicate that the arduous work environment of the WLFF is a challenge to the maintenance of energy balance, FFM and hydration in men and women. The observed decrease in total body weight may in part be explained by 1) changes in hydration status, 2) muscle glycogen, and/or 3) alterations to the protein component of the FFM.

Study III - Seasonal variations in total body water from multifrequency bioelectrical impedance and deuterium dilution in the wildland firefighter

Abstract

Previously, our lab has used the doubly labeled water methodology to demonstrate that the TEE of the Wildland Firefighter (WLFF) may range from 3500 to 7000 kcal/day (MSSE 30; S56). We have also shown that during short-term wildfire suppression (approx. 5-7 days), males can show a significant decrease in FFM (MSSE 32; S39). This research was undertaken to determine the changes in total body water (TBW), body weight (BW) and body composition (FFM, FBM) over an entire season of wildland fire suppression work in males and females. A group of type I WLFF (n=11 females, n=13 males) and a group of college aged controls (n=11 females, n=9 males) served as subjects. A combination of methods was used to determine TBW and body composition as follows: (1) Multifrequency bioelectrical impedance analysis (BIA) and deuterium (D_2O) for TBW (2) BIA, D_2O, and skinfolds (SKF) for body composition. There was a significant change in FFM for the male WLFF according to the BIA (pre=60.08 ± post=63.62 ±) and SKF (pre=70.10 ± post=71.52 ±). However, D_2O showed no significant changes in FFM (pre=63.35 ± post=65.45 ±). There was a significant change in FFM for the females according

to the BIA data (pre=43.53 ± post=45.88 ±). However, there were no changes in FFM calculated from the SKF (pre=52.65 ± post=53.45 ±) or D_2O (pre=48.41 ± post=48.54 ±) data. Although the female control subjects showed a significant increase in FFM according to the BIA (pre=39.13 post=40.00), there were minimal changes noted for the control group. There was a significant difference in TBW of the experimental group (males and females) between D_2O and BIA (p=.05). However, the difference was not consistent in the control group.

	BIA (FFM)		D_2O (FFM)		SKF (FFM)	
	pre	post	pre	post	pre	post
Males - EXP	60.08	63.62	63.35	65.45	70.10	71.52
CNTRL	65.45	66.46	64.65	64.28	71.74	71.53
Females - EXP	43.53	45.88	48.41	48.54	52.65	53.45
CNTRL	39.13	40.00	21.68	42.05	48.30	47.84

BIA consistently under estimates TBW compared to D_2O, which may be due to hydration issues or environmental testing conditions.

Introduction

Previous research has indicated that the short term (up to 10 days of work) energy demands during field operations can routinely exceed in the upwards of 5000-6000 $kcals \cdot 24^{-1}$ based on the use of doubly labeled water (Mudambo 97, Hoyt 91, Stroud 93). The majority of this research is conducted during military operations or during expedition (mountain or arctic). During the summer months in the western part of the United States, a variety of agencies (United States Forest Service, Bureau of Land Management, State Forestry) are involved in controlled burn operations and wildfire suppression.

Wildland fire suppression is a unique seasonal occupation (May – October in the West) that requires long hours of heavy work under adverse conditions (extended work shifts up to 24 hours, high ambient heat, compromised dietary intake, smoke inhalation, and altitude exposure). Based on a conservative estimate of energy expenditure for typical wildland firefighting tasks (7.5 $kcals \cdot min^{-1}$, 12-14 hour work shift), work shift energy expenditure may exceed 4050 to 4725 $kcals \cdot 12$ and 14 $hours^{-1}$, respectively (assuming 45 minutes of work each hour). Therefore, a simple job task analysis reveals that the energy demands of the job are extreme and represent a challenge to the maintenance of energy balance. This is especially true considering the length of the fire season and the possibility of working on several field assignments.

Our laboratory has recently determined the total energy expenditure during wildland fire suppression activities using the doubly labeled water and heart rate methodologies (Ruby 1999, Burks, 1998). Although there is variation in the calculated rates of TEE (dependent on fire location, work detail, amount of hiking and fire line construction), values range from approximately 3000 – 6500 $kcals \cdot 24\ hours^{-1}$ (Ruby, 1999). We have also demonstrated an acute loss of total body weight and fat free mass often accompanies a typical five-day work detail. These previous data demonstrate a unique work environment that results in an abrupt increase in the required dietary intake patterns. Wildland firefighters are required to "self-adjust" to the increase in TEE within the restrictions of what is provided for them in the fire camp.

The adequacy of common food rations on the maintenance of energy balance was investigated during 12 days of military operations in the heat (African bush) (Mudambo et. al, 1997). Using the doubly labeled water and energy balance methods, TEE was calculated (5489±358 and 6205±167 $kcals \cdot 24\ hours^{-1}$ for the DLW and EB methods, respectively). During the 12-day

period of combined heat stress and work, subjects lost 3.0±0.1 kg (from energy deficit and a decrease in total body water) indicating a deficiency in the standard food rations provided. Similar studies have demonstrated significant changes in energy balance in response to adverse field conditions in the cold (Delany, 1989), during progressive hypoxia during mountain expedition (Pulfrey, 1996; Westerterp, 2000) during extended training (Sjodin, 1994), and during space flight (Lane, 1997; Stein, 1999).

There is an inconsistent pattern within the previous research regarding an individuals ability to maintain energy balance during extreme field operations that result in TEE greater than 4,000 $kcal \cdot 24\ hours^{-1}$. However, the maintenance of energy balance is dependent on TEE and on the availability of foodstuffs in the field and consistent and adequate intake behaviors. Regardless of availability, sustained or suppressed appetite will enhance and/or impede the maintenance of energy balance when matched with arduous field conditions. This is especially important to consider given the nature of the occupation and the likelihood of accumulating over 1000 hours of overtime in a busy season.

Consequently, the purpose of this study was to determine the maintenance of energy balance and body composition in male and female wildland firefighters during the course of the fire season (five months). Based on our previous research that demonstrates an elevated short term total energy expenditure and a trend towards weight loss, we hypothesized that a season of wildfire suppression work would likely lead to further weight loss and changes in body composition.

Methods

Subjects

Subjects included wildland firefighters (N=24, 11 F, 13 M) recruited from four Interagency Hot Shot Crews (Lolo, Bitterroot, St. Joe, Sierra crews) from Western Montana, Idaho, and Northern California and recreationally active University students (N=20, 11 F, 9 M). Subjects were recruited through an informative meeting arranged between the Principal Investigator and all Interagency Hot Shot Crew Supervisors prior to the 1998 fire season. An informational meeting was then arranged between the Principal Investigator and the entire crew. At this time, the objectives of the study and the outline of data collection were discussed. Potential subjects were selected and were tested at their base operations center during the pre season training courses and after the season during the last week of employment. Control subjects were recruited through the undergraduate and graduate courses within the Department of Health and Human Performance. Control subjects were tested during a similar experimental period (five months) over the course of an academic semester. Testing for all control subjects was conducted at the human performance laboratory at the University of Montana.

Preliminary Screening

Prior to data collection, all subjects read and signed an Internal Review Board (IRB) approved human subject's consent. Subjects completed a detailed health history to determine prior exercise and training habits and menstrual regularity.

Total Body Water and Skinfold Measurements

At approximately 0630, subjects were provided with an oral dose of ^2H_2O (approximately 2 grams- Cambridge Isotope Laboratories, Andover, MA) after the collection of a background saliva sample. The ^2H_2O was mixed in 35 ml of tap water and was rinsed three times to ensure complete isotopic delivery. Subjects refrained from the consumption of food or additional water until additional saliva samples were collected at three and four hours post dose. A measure of nude body weight was obtained (accuracy ±100 grams). All subsequent voids were collected to correct the measure of total body water (TBW). Samples were collected and stored in 5 ml cryogenic vials on ice for the duration of the experimental period. TBW was calculated from the change in isotopic enrichment (background vs. the four hour sample) using equation 1. Control subjects were studied in the University laboratory on campus and reported for testing at similar time points for the background and dosing protocol and the collection of saliva samples (approximately 0600).

In addition to the measure of TBW and body composition from ^2H_2O dilution, skinfold measures were completed on each subject. Skinfolds were collected in rotational order according to the gender specific formulas of Jackson and Pollock (1978, 1981). No less than three independent site measurements were obtained until repeat measurements were within ±.1 mm. Because the original prediction equations of Jackson and Pollack (1978, 1981) were developed with the Lange skinfold calipers, an adjustment of +2mm for each site was included to compensate for differences noted for the Harpenden calipers (Golding et. al, 1989). Body density was converted to percent body fat using an appropriate age and gender equation of Lohman (1992). Body

composition was also estimated from the TBW values (calculated from ^2H_2O dilution, corrected for overnight void collections). Fat free mass (FFM) was calculated as TBW/.73. Fat body mass (FBM) was calculated from the difference in the nude body weight and the calculated FFM.

Total body water (TBW) was also measured with a multifrequecy bioelectrical impedance analyzer (Xitron, Inc., San Diego, CA). No more than 30 minutes prior to the measure of TBW, subjects urinated. Subjects adhered to all pre-test guidelines (caffeine, alcohol, physical activity) prior to the measurement. Two electrodes were placed on the right hand and wrist (10 cm separation) and at the right ankle and foot (12 cm separation) after cleansing each site with alcohol. Measurements were performed after subjects remained supine for 10 minutes. At minute 9, the start sequence was initiated and the measurement was completed by minute 10. The same testing environment was retained pre and post season for all subjects (laboratory or base operations center). TBW was expressed in liters and was converted to kg by considering the density of water at body temperature (TBW (L)/.9937 = TBW(kg)). FFM and FBM was calculated from the TBW assuming that the FFM had a water content of 73% (FFM (kg) = TBW/.73). FBM was calculated from the nude body weight and (FBM = FFM – TBW).

A second dose of ^2H_2O was provided at the end of the season following the collection of an additional background saliva sample. The same procedures for isotopic dosing, sample collection, nude weight, skinfold, and BIA measures were completed at this time to evaluate post season changes in body composition and TBW. Control subjects underwent the identical procedures with the exception of wildfire suppression.

Isotopic analyses

The Nutritional Sciences Laboratory at the University of Wisconsin, Madison, conducted isotopic analyses of all urine samples. Breifly, each sample was mixed with ca. 200 mg of dry carbon black and filtered through a 0.22 micron filter to remove particulate materials and much of the organic material. Two 1mL aliquots of each specimen were placed in 2 mL septum sealed, glass vials. Deuterium analysis was performed by reducing 0.8uL of cleaned fluid over chromium at 850°C (Gehre et. al, 1997), which produces pure H_2 gas that is introduced to a Finnigan MAT Delta Plus isotope ratio mass spectrometer. Deuterium abundance was measured against a working standard using a standard dual inlet, Faraday Cup, differential gas isotope ratio procedure. Enriched and depleted controls were analyzed at the start and end of each batch and these secondary standards used to calculate the "per mille" abundance versus Standard Mean Ocean water for each urine sample. All analyses were performed in duplicate and all specimens from the same participant analyzed during the same batch. Results were corrected for any memory from the previous chromium reduction process. If duplicates differed by more than 5 per mil, duplicate analyses were repeated. The second aliquot was equilibrated with 1 mL (STP) of carbon dioxide at constant temperature (Schoeller, 1997). The CO_2 was removed by syringe and roughly 200 uL injected onto a 10 cm x 1/8" Chromasorb Q column. The CO_2 peak was introduced into the ion source of a Finnigan MAT Delta S isotope ratio mass spectrometer and the $^{18}O/^{16}O$ ratio measured under dynamic flow conditions. A secondary standard was injected at the start and end of each batch. The secondary standard was used to calculate the "per mille" abundance versus Standard Mean Ocean Water (SMOW) for each specimen. Analyses were performed in duplicate and all specimens from the same participant analyzed during the same

batch. Results were corrected for any memory from the previous chromium reduction. If duplicates differed by more than 0.5 per mil, analyses were repeated in duplicate.

Isotope dilution space was calculated as described by Coward and Cole (1992). Total body water was calculated by averaging the deuterium dilution space/1.041 (see Equation 1 below).

Equation 1. Calculation of total body water from the change in isotopic enrichment .

$$\left[\text{TBW (kg)} = \frac{d}{MW} \bullet \frac{APE}{100} \bullet \frac{18.01}{R_{std} \bullet \Delta\delta^2} \right] \Big/ \quad 1.041$$

d =	isotopic dose in grams
MW =	the molecular weight of ^2H_2O (20.00)
APE =	atom percent excess of ^2H_2O stock solution (99.99)
18.01 =	molecular weight of unlabelled water
R_{std} =	isotopic difference noted in the standard (0.00015576)
$\Delta\delta^2$ =	change in enrichment from background (relative to SMOW) to second void
1.041 =	assumed isotope dilution space for ^2H_2O.

Statistical Methods

Each dependent variable was analyzed across the season (pre vs. post) for males and females using a dependent two tailed t-test. Pooled data (M+F) were used to determine differences in calculated TBW across the two methods (BIA and D_2O dilution). All data are expressed as mean±sd.

Results and Discussion

Seasonal changes in body composition for the experimental subjects (wildland firefighters) are reported in Table III-1. There were no statistically significant changes in total body weight for

the male or female subjects. Males demonstrated a significant increase in FFM as determined by skinfold and BIA. In contrast, females only the BIA demonstrated a significant increase in FFM for the female subjects. Males demonstrated a significant decrease in FBM according to all three methodologies. In contrast, FBM remained unchanged in the females according to the three measures.

Seasonal changes in body composition for the control subjects are reported in Table III-2. Males did not show a significant change in BW, FFM or FBM for either method across the measurement period. Females did not show a significant change in BW. Females did demonstrate a significant decrease in FFM according to the BIA measure. Females also showed a significant increase in FBM according to the skinfold measure. FFM and FBM remained unchanged according to the other measures.

Table III-1. Experimental group data. Changes in energy balance related variables for the males (n=13) and females (n=11) during the seasonal experimental period. Data is represented as mean±sd.

Variable	MALES		FEMALES	
	Pre-season	Post- season	Pre- season	Post- season
Nude Body Weight (kg)	80.3±12.2	80.4±12.2	64.8±6.1	64.6±7.4
FFM (skinfold – kg)	70.1±7.4	71.5±7.9*	52.7±3.0	53.5±4.4
FBM (skinfold – kg)	10.2±6.6	8.9±5.8*	12.1±3.4	11.2±3.8
FFM (BIA – kg)	60.1±7.6	63.6±8.1*	43.5±4.0	45.9±4.2*
FBM (BIA – kg)	20.3±7.0	16.8±7.6*	21.2±3.1	18.7±4.3
FFM (D_2O – kg)	63.4±7.0	65.5±9.3	48.4±2.9	48.5±3.5
FBM (D_2O – kg)	17.0±7.0	15.0±7.4*	16.4±4.0	16.1±5.2

*p<0.05 vs. pre season

Table III-2. Control group data. Changes in energy balance related variables for the males (n=9) and females (n=11) during the seasonal measurement period. Data is represented as mean±sd.

Variable	MALES		FEMALES	
	Pre-season	Post- season	Pre- season	Post- season
Nude Body Weight (kg)	80.4±10.1	80.4±12.2	61.3±8.3	61.7±8.5
FFM (skinfold – kg)	70.7±8.8	71.5±10.0	48.3±5.4	47.8±5.2
FBM (skinfold – kg)	8.7±4.1	8.9±4.3	13.0±3.8	13.8±4.1*
FFM (BIA – kg)	65.5±7.9	66.5±10.8	39.1±5.0	40.0±5.1
FBM (BIA – kg)	15.0±3.7	14.0±3.9	22.2±5.5	21.7±5.3*
FFM (D_2O – kg)	64.6±8.7	64.3±9.3	42.1±4.3	42.7±4.9
FBM (D_2O – kg)	15.8±4.1	16.1±5.5	19.2±4.9	19.0±4.9

*$p<0.05$ vs. pre season

Changes in total body water and differences across the BIA and D_2O methodologies are reported in Tables III-3 and III-4 for the experimental and control groups, respectively. For the experimental group, the males and females demonstrated a significant increase in TBW as measured by the BIA. There were no significant differences across the season according to the D_2O data. The BIA consistently demonstrated significantly lower values for TBW compared to the D_2O dilution method with the exception of the male post-season time point. For the control group, the females demonstrated a significant increase in TBW according to the BIA measure. However, there were no differences noted in the males. According to the D_2O data, there were no changes in TBW for either the male or female control subjects over the measurement period. The BIA consistently demonstrated significantly lower values for TBW compared to the D_2O dilution method in the females. However, there were no significant differences across the two methods in the males.

Considering the overall means, the experimental group demonstrated a significant difference between the two methods at the pre and post time points. In contrast, the control group did not show a difference in TBW between the BIA and D_2O methods. Correlational data for each group are presented in figures III-1 and III-2. Although there is a significant correlation between the two methods for the experimental group, BIA consistently underestimated TBW in comparison to the D_2O dilution method. In contrast, the control group demonstrated a significant correlation between methods with no significant difference in the calculated TBW for either time point.

Table III-3. Experimental group data. Methodological comparisons for the measure of TBW for the males (n=13) and females (n=11) during the seasonal experimental period. Data is represented as mean±sd.

Variable	MALES		FEMALES	
	Pre-season	Post- season	Pre- season	Post- season
D_2O TBW (kg)	46.56±5.1#	48.1±6.8	35.6±2.1#	35.7±2.5#
BIA TBW (kg)	44.2±5.6	46.8±6.0*	32.0±3.0	33.7±3.1*

Overall Means

	Pre-season	Post- season
BIA TBW (kg)	38.6±7.6	40.8±8.2*
D_2O TBW (kg)	41.5±6.9#	42.4±8.2#

*p<0.05 vs. pre season, # p<0.05 vs. BIA

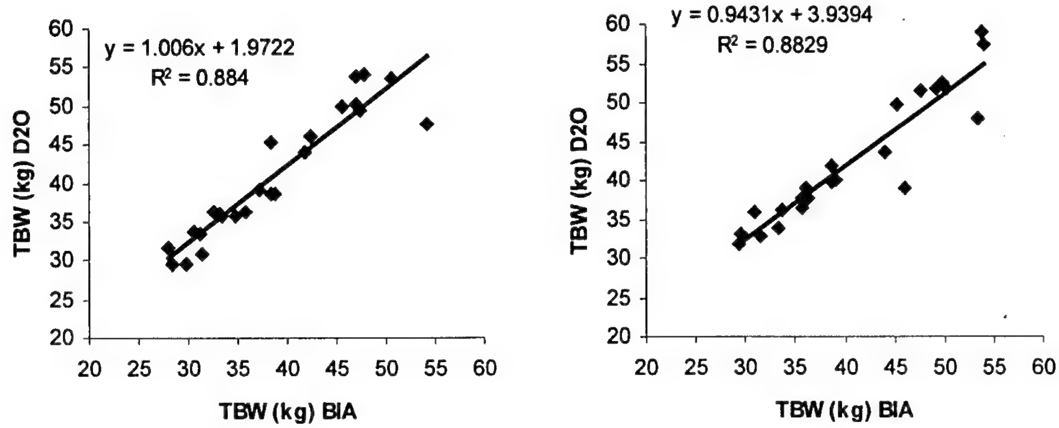
Table III-4. Control group data. Methodological comparisons for the measure of TBW for the males (n=13) and females (n=11) during the seasonal measurement period. Data is represented as mean±sd.

Variable	MALES		FEMALES	
	Pre-season	Post- season	Pre- season	Post- season
D_2O TBW (kg)	47.5±6.4	47.2±6.8	30.9±3.2#	31.4±3.6#
BIA TBW (kg)	48.1±5.8	48.8±8.0	28.8±3.6	29.4±3.7*

Overall Means

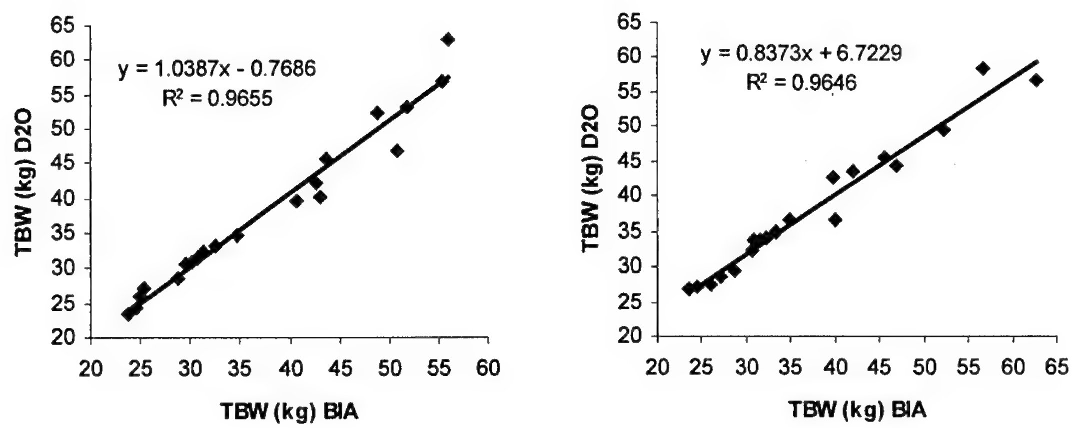
	Pre-season	Post- season
BIA TBW (kg)	37.5±10.9	38.2±11.5
D_2O TBW (kg)	38.4±9.7	38.5±9.6

*p<0.05 vs. pre season



Pre Experimental Period **Post Experimental Period**

Figure III-1. Relationship in calculated TBW between BIA and D_2O dilution for the pre and post time points in the experimental group.



Pre Experimental Period **Post Experimental Period**

Figure III-2. Relationship in calculated TBW between BIA and D_2O dilution for the pre and post time points in the control group.

Discussion:

These results indicate that the overall seasonal stress is considerably different compared to an acute wildfire suppression assignment. It is not unusual for crews to be deployed and work

extremely hard on a fire assignment for up to 21 days and then have an entire month of limited project work. It is apparent that although the acute stress is extreme, the overall effects of the season are comparable to a structured exercise program. If the dietary restrictions/limitations were consistent throughout the season, decreases in FFM may have occurred. In contrast, there was a general trend towards an increase in the FFM and a decrease in the FBM of the male and female firefighters.

It is important to note that when subjects are not on a wildfire assignment, they live in their own homes and apartments in the community and have all the dietary advantages associated with that. They are free to self-select their own intake and physical activity patterns. If there is a change that may occur on a week-long fire assignment due to inadequate dietary intake, there is an obvious adjustment during the time not on fire assignment.

These data are unique in that they suggest that the wildland firefighter can make the necessary adjustments over the course of the season to maintain body weight and FFM. However, as indicated in investigation IV, it is difficult for the subjects to make the necessary adjustments while on the fireline. It is unclear whether this is a function of the increased daily energy expenditure or due to an overall reduction in the variety of food stuffs. Some subjects appear to be able to make the necessary adjustments and limit FFM loss, whereas others are unable to adjust and lose FFM. We did not collect dietary habit questionnaire to determine dietary selection traits that may predispose a subject to weight loss when they are unable to self-select their own food choices. It should also be considered that the subjects included in the study were experienced wildland firefighters. As HotShot crew members, most had accumulated a number

of years of wildfire experience and were accustomed to the fire camp diet. These subjects were also relatively active and passed the current wildfire fitness standard established by our laboratory. This fitness standard requires a subject to complete a 3 mile flat walk/hike with a 45 lb pack averaging 4 miles/hour or less (total time = 45 minutes). The estimated oxygen consumption of this activity is approximately 22.5 ml/kg/min under the assumption that the wildland firefighter must be able to work at exercise intensities equivalent to 50% of maximal oxygen uptake. All subjects in the current study passed the fitness standard indicating a relatively high level of fitness.

These data indicate that as long as subjects can return to their own self-selected dietary and physical activity patterns during times not on wildfire assignment the changes in body weight and composition are similar to a regular exercise program. However, each fire season is different and determined by the weather the previous year and seasons. Therefore, it is feasible to have an extremely slow season similar to that of 1997 with less than four wildfire assignments. It is also feasible to have an intense fire season resulting in multiple 21-day work cycles*. Therefore, it is important to offer subjects a variety of food sources during these extended work cycles to maintain body composition and to limit FFM loss.

*The Interagency HotShot crew can be on wildfire assignment up to 21 days straight without a break. Following the 21 day period, there is a mandatory two day rest period that may or may not occur at home. Often crews are put in Hotels in a nearby community for the mandatory two day rest.

III. Conclusions

Overall, these series of investigations have developed the use of a unique physiological human model that allows the evaluation of the subject in a non-simulated arduous work cycle. The benefit of this model is that the occupation of wildland fire suppression involves many of the rigors similar to warfare that include the physical and psychological stress that are difficult to simulate in other models. Future research should further develop this model and use a variety of additional methodologies to evaluate the unique physiological stresses that may affect males and females.

Future research should continue to evaluate the following areas with the use of this unique physiological model.

- The nutritional issues surrounding energy balance
- Gender differences in the maintenance of blood glucose during arduous occupational stress
- Changes in muscle glycogen associated with arduous occupational stress over 5-7 days
- Immune function and nutritional strategies to minimize fatigue and sickness during arduous operations
- Effects of arduous operations on the oxidative stress profiles of males and females
- Evaluation of total protein turnover rates during arduous operations using ^{15}N-glycine and ^{15}N-alanine to determine the rationale for FFM loss.

Regardless of the additional questions that may be proposed using this current physiological model, it is critical that the effects of gender be included. This is especially true in light of the data presented in this report. It should not be assumed that the biological and physiological differences between males and females are not important to consider when personnel are placed

in an arduous operation. Regardless, this is the assumption that has been historically maintained in the literature. Our data conclusively suggests that males and females respond differently to arduous physical stress (i.e. weight loss, fuel selection, nutritional intake, hydration and bone metabolism). For this reason, further research should work to maximize what we know about these sex specific responses so as to increase the overall health care strategy for the combat soldier or any other occupation that may involve extreme physiological stresses.

References

1. Blomstrand, E. and B. Saltin. Effect of muscle glycogen on glucose, lactate and mino acid metabolism during exercise and recovery in human subjects. *J. Physiol.* 1: 293-302, 1999.

2. Burstein, R., A.W. Coward, W.E. Askew, K. Carmel, C. Irving, O. Shpilberg, D. Moran, A. Pikarsky, G. Ginot, M. Sawyer, R. Golan, and Y. Epstein. Energy expenditure variation in soldiers performing military activities under cold and hot climate conditions. *Mil. Med.* 161: 750-754, 1996.

3. Burks, C.A., B.J. Sharkey, S.A. Tysk, T.W. Zderic, S.L. Johnson, and B.C. Ruby. Estimating energy expenditure in wildland firefighters using heart rate monitoring and physical activity records. *Med. Sci. Sports Exerc.* 30: s56, 1998.

4. Coward, A. and T. Cole. Precision and accuracy of the doubly labeled water energy expenditure by multipoint and two-point methods. *Am . J. Physiol*l. 263:E965-E973, 1992.

5. Delany, J.P., D.A. Schoeller, R.W. Hoyt, E.W. Askew, and M.A. Sharp. Field use of D2 18O to measure energy expenditure of soldiers at different energy intakes. *J. Appl. Physiol.* 67: 1922-1999, 1989.

6. Forbes-Ewan, C.H., B.L. Morrissey, G.C. Gregg, and D.R. Waters. Use of doubly labeled water technique in soldiers training for jungle warfare. *J. Appl. Physiol.* 67:14-18, 1989.

7. Gehre, M, R. Hoeflin, P. Kowski, and G. Stauch. Sample preparation devise for quantitative hydrogen isotope analysis using chromium metal. *Anal . Chem.* 1997.

8. Golding, L.A., C.R. Myers, and W.E. Sinning. Y's way to physical fitness, 3rd edn. Human kinetics. Champaign, Ill, p85, 1989.

9. Hoyt, R.W., T.E. Jones, T.P. Stein, G.W. McAninich, H.R. Lieberman, E.W. Askew, and A. Cymerman. Doubly labeled water measurement of human energy expenditure during strenuous exercise. *J. Appl. Physiol.* 71: 16-22, 1991.

10. Hoyt, R.W., T.E. Jones, CJ. Baker-Fulco, D.A. Schoeller, R.B Schoene, R.S. Schwartz, E.W. Askew, and A. Cymerman. Doubly labeled water measurement of human energy expenditure during exercise at high altitude. *Am. J. Physiol.* 35: R066-R971, 1994.

11. Jackson, A.S. and M.L. Pollock. Generalized equations for predicting body density of men. *Brit. J. Nutri.* 40: 497-504, 1978.

12. Jackson, A.S., M.L. Pollock and A. Ward. Generalized equations for predicting body density in women. *Med. Sci. Sports Exerc.* 12: 175-182, 1980.

13. Lohman, T. G. Advances in Body Composition Assessment. Current issues in exercise in exercise science series. Monograph No. 3. Champaign, IL: Human Kinetics, 1992.

14. Jones, P.J., I. Jacobs, A. Morris, and M.B. Ducharme. Adequacy of food rations in soldiers during an arctic exercise measured by doubly labeled water. *J. Appl. Physiol.* 75: 1790-1797, 1993.

15. Lane, H.W., R.J. gretebeck, D.A. Schoeller, J. Davis-Street, R.A. Socki, and E.E. Gibson. *Am.. J. Clin. Nutr.* 65: 4-12, 1997.

16. Mudambo, K.S., C.M. Scrimgeour, and M.J. Rennie. Adequacy of food rations in soldiers during exercise in hot, day-time conditions assessed by doubly labeled water and energy balance methods. *Eur. J. Appl. Physiol.* 76:346-351, 1997.

17. Pulfrey, S.M. and P.J. Jones. Energy expenditure and requirement while climing above 6,000 m. *J. Appl. Physiol.* 81: 1306-1311, 1996.

18. Ruby, B.C., T.W. Zderic, C.A. Burks, S. Tysk, and B.J. Sharkey. Total energy expenditure (doubly labeled water) and bone resorption during wildland fire suppression. *Med. Sci. Sports Exerc.* 31: s366, 1999.

19. Schoeller, D.A. and A.H. Luke. Rapid ^{18}O analysis of CO_2 samples by continuous flow isotope ratio mass spectrometry. *J. Mass Spectrom.* 32:1332-1336, 1997.

20. Schoeller, D.A. Recent advances of doubly labeled water to measurement of human energy expenditure. *J. Nurtr.* 129: 1765-1768, 1999.

21. Sjodin, A.M., A.B. Andersson, J.M. Hogberg, and K.R. Westerterp. Energy balance in cross-country skiers: a study using doubly labeled water. *Med. Sci. Sports Exerc.* 26: 720-724, 1994.

22. Stein, T.P., M.J., Leskiw, M.D. Schluter, R.W. Hoyt, H.W. Lane, R.E. Gretebeck, and A.D. LeBlanc. Energy expenditure and balance during spaceflight on the space shuttle. *Am. J. Physiol.* 276: R1739-R1748, 1999.

23. Stroud, M.A., W.A. Coward, and M.B. Sawyer. Measurements of energy expenditure using isotope-labeled water ($^{2}H_2{}^{18}O$) during an arctic expedition. *Eur. J. Appl. Physiol.* 67: 375-379, 1993.

24. Trappe, T.A., A. Gasteldelli, A.C. Jozsi, J.P. Troup, and R.R. Wolfe. Energy expenditure of swimmers during high volume training. *Med. Sci. Sports Exerc.* 29: 950-954, 1997.

25. Westerterp, K.R., W.H.Saris, M. van Es, and F. ten Hoor. Use of the doubly labeled water technique in humans during heavy sustained exercise. *J. Appl Physiol.* 61: 2162-2167, 1986.

26. Westerterp, K.R., B. Kayser, F. Brouns, J.P. Herry, and W.H. Saris. Energy expenditur climbing Mt. Everest. *J. Appl. Physiol.* 73:1815-1819, 1992.

27. Westerterp, K.R., B. Kayser, L. Wouters, J.L Le Trong, and J.P. Richalet. Energy balance at high altitude of 6,542 m. *J. Appl. Physiol.* 77: 862-866, 1994.

28. Westerterp, K.R. Body composition, water turnover and energy turnover assessment with labelled water. *Proc. Nutr. Soc.* 58: 945-951, 1999.

29. Westerterp, K.R., E.P. Meijer, M. Rubbens, P. Robach, and J.P. Richalet. Operations Everest III: energy and water balance. *Pflugers Arch.* 439: 483-488, 2000.

30. Wolfe RR. Radioactive and stable isotope tracers in biomedicine. *Principles and Practice of Kinetic Analysis* . New York:Wiley-Liss, 1992.